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REVIEW ARTICLE

Pathogenesis, Experimental Models and Contemporary Pharmacotherapy of Irritable Bowel Syndrome: Story About the Brain-Gut Axis

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Abstract: ***Background***: Although the precise pathophysiology of irritable bowel syndrome (IBS) remains unknown, it is generally considered to be a disorder of the brain-gut axis, representing the disruption of communication between the brain and the digestive system. The present review describes advances in understanding the pathophysiology and experimental approaches in studying IBS, as well as providing an update of the therapies targeting brain-gut axis in the treatment of the disease.

Methods: Causal factors of IBS are reviewed. Following this, the preclinical experimental models of IBS will be introduced. Besides, both current and future therapeutic approaches of IBS will be discussed.

Results: When signal of the brain-gut axis becomes misinterpreted, it may lead to dysregulation of both central and enteric nervous systems, altered intestinal motility, increased visceral sensitivity and consequently contributing to the development of IBS. Interference of the brain-gut axis can be modulated by various psychological and environmental factors. Although there is no existing animal experiment that can represent this complex multifactorial disease, these *in vivo* models are clinically relevant readouts of gastrointestinal functions being essential to the identification of effective treatments of IBS symptoms as well as their molecular targets. Understanding the brain-gut axis is essential in developing the effective therapy for IBS. Therapies include improvement of GI motor functions, relief of visceral hypersensitivity and pain, attenuation of autonomic dysfunctions and suppression of mucosal immune activation.

Conclusion: Target-oriented therapies that provide symptomatic, psychological and physiological benefits could surely help to improve the quality of life of IBS patients.

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INTRODUCTION

Irritable bowel syndrome (IBS) represents one of the most commonly occurred functional disorders of the gut that affects around 11% of the global population [1]. The symptoms perceived in IBS patients include abdominal pain or discomfort that is associated with alteration of either consistency or frequency of stools free from gross abnormalities [2]. In 1978, Manning's group established six criteria to distinguish IBS from organic bowel diseases [3]. Later on, Talley and his team further revealed that the Manning criteria are applicable in 58% and useful in 74% of the clinical cases that excluded IBS from organic gastrointestinal (GI) diseases [4]. These criteria were eventually reorganized in 1999 and the Rome criteria have now been adopted as the recognized criteria for both research studies and clinical practice [5]. According to the Manning and Rome II diagnostic criteria, IBS is defined as "abdominal pain and alteration of bowel habits". The Rome criteria represent a positive predictive value of roughly 98% of the cases, while supplementary diagnostic tests have a yield of 2% or less [6]. Yet, many investigations have pointed to the fact that the diagnostic criteria for IBS need further verification [7]. It should be noted that the estimates of prevalence can be variable simply because of the deviations between different epidemiologic studies, such as the use of diagnostic criteria, population selection and source of data.

Based on the clinical presentation of constipation, diarrhea, or their combination, IBS can be categorized in three major subtypes, including "constipation-predominant IBS" (IBS-C), "diarrhea-predominant IBS" (IBS-D) and "mixed IBS" (IBS-M) [2]. A brief summary on the subtyping of IBS has been listed in Table **1**. It was reported that about 1/3 of patients possess IBS-D, 1/3 having IBS-C and the remaining 1/3 have IBS-M. The categorization of IBS

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Table 1. Subtypes of IBS in terms of predominant stool pattern.

Subtype	Abbreviation	Stool Pattern
IBS with constipation	IBS-C	Infrequent stools, hard or dry stools, straining, bloating, difficulty at stools
IBS with diarrhea	IBS-D	Frequent stools, loose or watery stools
Mixed IBS	IBS-M	Both patterns of constipation and diarrhea
Alternating IBS	IBS-A	Shifting patterns of constipation and diarrhea
Un-subtyped IBS	IBS-U	Insufficient characters of a specific type of IBS

patients into different subtypes is essential in clinical practice for effective treatments according to differential symptoms. However, it is common for IBS patients to switch among different subtypes, in which case categorized as "alternating IBS" (IBS-A) [8]. Nevertheless, un-subtyped IBS occurs occasionally when insufficient characters of a specific type of IBS of the patients can be identified. Treatment options include pharmacological symptomatic relief of the associated pain, diarrhea or constipation. More than 40% of patients have severe symptoms, leading to reduction in the quality of life (QoL) and frequent visits to the clinic. Even with the differences in social and cultural environments between the East and West, the results concerning the QoL (both mental and physical) in Asian population are in agreement with those studies conducted in the West [9]. It is known that patients with IBS-C represent a substantial economic burden to both patients and their employers. The total cost difference for IBS patients compared with matched controls has been estimated to be US$1,896 in 2010 [10]. In addition, IBS could be diagnosed twice more frequently in women than in men. Nevertheless, studies have demonstrated that the occurrence of pain-related symptoms of IBS are equal between men and women, while the occurrence of symptoms not related to pain including constipation, bloating and extra-intestinal manifestations would be more commonly found in women [11]. Any disruption and alteration of the brain-gut axis can be regarded as etiological factor of IBS [12]. The present review attempts to provide update information on the pathogenesis and pre-clinical experimental models of IBS, and to discuss on the contemporary pharmacotherapy and alternative approaches directed at the brain-gut axis in the treatment of IBS.

PATHOPHYSIOLOGIC FEATURES OF IBS

The pathophysiology of IBS remains uncertain as no single physiologic mechanism has been identified for IBS [13]. Recent studies have suggested a significant association between the gut and central nervous system control. Thus, IBS has currently been viewed as a biopsychosocial disorder caused by the interaction among visceral hyperalgesia, genetic and environmental factors, infection, inflammation, gut motility, and other psychological conditions [14]. Data obtained from numerous investigations have been conflicting and no abnormality was found to be specific for this disorder. The traditional thought on the disease has been focused on alterations in gastrointestinal motility and on visceral hypersensitivity. There are three main patho-physiological mechanisms associated with IBS: psychosocial modulation, altered motility and altered sensation [15]. The development of IBS could be resulted from psychological stress, food intolerance or allergy, intestinal infection or injury, intestinal immune disruption and/or inflammation, changes in the intestinal microbiota or bacterial overgrowth, genetics, as well as abuse and early-life learning [16]. More recent studies have begun to consider the role of inflammation, alteration in fecal flora, and bacterial overgrowth in IBS development. In general, the pathogenesis of IBS appears to be multifactorial. The relationship between IBS and visceral hypersensitivity has been studied and well described. This review will mainly focus on the psychological factors, infection and inflammation, and the brain-gut interaction.

Diet

Diet could be an important factor to trigger IBS development. Although the contribution of gluten (wheat products in general) to IBS remains debatable, there has been a strong correlation with non-celiac gluten sensitivity in IBS patients. Nevertheless, it is more imminent that IBS symptoms are triggered by consumption of poorly absorbed fermentable oligo-, di-, monosaccharides and polyols (FODMAPs) as well as insoluble fibers [17]. This is the reason why dietary guidance for intake of poor FODMAPs and insoluble fibers is to be recommended for IBS patients. Other than food allergy, the impact of food processing may also be considered. In a study of the effects of breads with different lengths of fermentation on the colonic microbiota, it was found that bread fermented by the traditional long fermentation (as in commercial yeasted dough) and by the sourdough process (for 8 hours) are less likely to lead to IBS symptoms when compared to bread made by using the Chorleywood bread-making process (with no time fermentation), as determined by the amount of cumulative gas being produced in the gut of both healthy and IBS patients [18]. This is probably due to the fact that IBS patients have an imbalanced microbiota in the gut, while the bread-making process will influence gut microbial fermentation.

Psychological Factors

IBS has been identified as a psychosomatic condition because it does not have a single cause or pathophysiology. The medical term "stress" was first defined by the

endocrinologist Hans Selye in 1936 as "the physiological adaptive responses to perceived (psychological) or real (physical) threats ("stressors") to an organism" [19]. Psychological stress and emotional events such as physical or sexual abuse can cause gastrointestinal symptoms in both healthy subjects and IBS patients, but the extent of influences will be greater in IBS patients. Depression, somatization, anxiety, hostility, phobia, and paranoia are the most common psychological symptoms that are associated with IBS. The percentage of IBS patients that meet the criteria for psychiatric diagnosis is up to 50% while the percentage patients with organic GI disorders and control subjects are only 20% and 15%, respectively [14]. Even though there are no specific psychological or psychiatric disorders that may directly correlate with IBS, identification of such disorders can help to assign appropriate psychological or psychopharmacological treatment regimens. Up till now, there are still conflicting evidences on the association between stress and the severity of IBS. Experimental studies have revealed that mucosal mast cells could be activated after acute stress, with migration to enteric nerves following chronic stress [20, 21]. These mast cells would in turn release neuropeptides such as 5-hydroxytryptamine (5-HT) and pro-inflammatory cytokines, which are the known mediators responsible for the altered intestinal sensation, motility, secretion and permeability characteristics of IBS [22]. Any disturbance of the intestinal barrier may cause the release of acetylcholine, glucocorticoids and corticotrophin-releasing hormone, together with activation of intestinal mast cells [23]. Other than mast cells, increased number of immune cells (*e.g.* T-lymphocytes) with concurrent production of various cytokines has been observed in the intestinal mucosa, which may play a role in the immunomodulation in IBS. The resulting dysfunction in the intestinal barrier could then result in local or systemic inflammatory reactions and activation of immune responses, which together leading to abnormality of GI functions. Thus, it is well accepted that low-grade inflammation, activated innate and adaptive immune responses are all involved in the pathogenesis of IBS [24, 25]. It has also been proposed that immune activation can be mediated by psychological stress and the altered body responses to stress in IBS patients. In this respect, the treatment for IBS can be focused on the management of stress and stress-induced body responses. Since conventional drug treatments by using laxatives and secretagogues was found to be relatively ineffective, non-pharmacological treatment approaches have become a new direction of research.

Infection and Inflammation

Bacteria are present in the normal gut, especially in lower parts of the intestine. IBS and small intestine bacterial overgrowth might share similar symptoms and their correlation has been studied. A previous investigation showed that 157 out of 202 (78%) IBS patients had small bowel bacterial overgrown, while intraepithelial lymphocytes, CD3 and CD25 cells in the lamina propria, neutrophils, and mast cells were increased accordingly [26]. Up to now, the exact mechanism governing the inflammatory changes remains unclear. The role of immune activation in the pathogenesis of IBS has been revealed in a study by investigating the mechanisms of post-infectious-IBS (PI-IBS) [27]. It is believed that about 10% of the IBS cases would be resulted from prior infection. About 6-17% of IBS patients who had undergone previous episodes of infectious gastroenteritis were affected [28]. About 1/4 of these patients showed persistent disturbance of bowel habit at 6 month (*e.g.* with increased stool frequency), with most of them recovered rapidly from bacterial gastroenteritis thereafter. The risk factors involved in developing PI-IBS include prolonged initial illness, toxicity from infectious microbes, smoking, mucosal inflammatory markers, female gender, depression, hypochondriasis and recent adverse life events. The mechanisms causing PI-IBS are still unknown but could involve residual inflammation, enterochromaffin and mast cells, enteric nerves, and gastrointestinal microbiota [29]. It has been reported that when compared to those without IBS-type symptoms, fecal calprotectin was significantly elevated in ulcerative colitis and Crohn's disease patients with symptoms of IBS, implying the presence of occult inflammation [30]. However, it is still largely unknown whether immune activation in IBS patients is largely dependent on infectious gastroenteritis and/or psychological stress [31].

Brain-Gut Interaction

It is generally accepted that there are two components of dysregulation in IBS: the dysregulation of motor nerves (involving the regulation of gastrointestinal smooth muscle contraction) and dysregulation of the sensory nerves (involving the linkage between the intestinal receptors and nerve endings to the CNS). Consequently, the abnormal intestinal motility, enhanced awareness and hypersensitivity to abdominal distension, contraction and discomfort are resulted [14]. The brain-gut axis constitutes the enteric nervous system, the gut wall, the central nervous system including the hypothalamic-pituitary-adrenal (HPA) axis [32]. Under physiological conditions, signals from the GI tract impact the brain and hence exerting the changes in motility, secretion, and immune function [33]. The brain-gut axis has been regarded as an important network for regulation of ingestion, digestion, gut proprioception, and peristaltic control of the gut. Any disruption in the structure and function of the brain-gut axis may deteriorate the perceptual and reflexive responses of the nervous system and hence leading to IBS or related GI disorders [34]. Visceral hypersensitivity is one of the main symptoms of IBS that has been determined by central and peripheral mechanisms resulting from altered neurotransmission of the enteric plexus, the spinal cord or the central nervous system [35]. Despite the unknown role of the brain-gut axis in the hypersensitive responses of IBS patients, the sensory, striatal, limbic, and frontal areas of the brain have manifested differential central responses to the distension of the colon among healthy subjects and IBS patients. It appeared that IBS patients exhibited a more profound activation of the anterior cingulate cortex, indicating the enhanced affective responses to painful visceral stimuli [36]. Furthermore, a previous study had also demonstrated that there is strong association between the central pathways mediating stress/anxiety and other mechanisms involving regulation of GI sensitivity in animals [37]. In addition, a

recent study demonstrated that abnormal hippocampal glutamatergic neurotransmission has been detected in IBS patients, but not in healthy individuals, with an inverse correlation between glutamate-glutamine concentrations and emotional stress indicators being observed [38].

PRECLINICAL EXPERIMENTAL MODELS OF IBS

Since the pathophysiology of IBS in human remains incompletely understood, a number of *in vivo* models have been developed for studying the different conditions of the IBS-related GI dysfunctions. In most occasions, laboratory animals, particularly rodents, are used as the subjects of the experimental models in preclinical studies [39]. Amongst these models, different kinds of stressors, for instance, psychological and physical stimuli as well as inflamogen, appear to play critical roles in the development of IBS and the maintenance of the disease. These models have been proposed to resemble either the entire disease process or cardinal features of this heterogeneous disorder (Fig. **1**). Moreover, owing to the advancement in biotechnology, several transgenic mouse strains have been established to mimic certain aspects of the human clinical syndromes. With the aid of validated experimental models, researchers are allowed to explore the distinctive pathophysiology of different subtypes of IBS, and to elucidate the mechanism of action of various prophylactic and/or therapeutic strategies against environmental, physiological and psychological factors [40]. It is generally understood that there is no ideal experimental animal model that exists to fully represent the complexity of multifactorial disorders such as IBS or inflammatory bowel disease (IBD) [41]. Nevertheless, these *in vivo* models are clinically relevant readouts of gastrointestinal functions and are essential to preclinical studies of IBS, especially the identification of successful treatments of IBS symptoms as well as their molecular targets [42]. The followings are some commonly used experimental animal models for pharmacological studies.

Neonatal Maternal Separation

According to some clinical studies, patients with life-threatening and/or stressful experience, such as loss or separation of family members, during childhood had an increased risk of IBS development in their adulthood [43]. In this regard, a clinically relevant animal model, in which puppies were separated from their mother periodically during postnatal period, had been established for the investigation of IBS induced by childhood insult [44, 45]. The neonatal maternal separation (NMS) procedures varies according to different duration periods, for instance, 1-4 hours of separation, as well as the number of separation episodes, such as 1-14 days after birth. In this model, the NMS rodents were demonstrated to develop visceral hypersensitivity in their adult life [46, 47]. Even with subtle alterations during their neonatal period, long-term consequences were found in puppies with respect to behavioural, emotional and stress responsiveness. In general, the neonatal period is considered as a critical time that exhibits great plasticity for both central and peripheral nervous systems as the HPA axis is programmed by early-life events [48]. Prolonged or consistent stress, such as maternal separation, may lead to altered development of the sensory system and distorted neuronal signalling pathways [49]. The outcome of this model has been suggested by large a dysregulated HPA reactivity [50]. Those altered nociceptive pathways may give rise to functional disorders in the somatic systems, particularly for stress responsiveness. Although there are distinctive differences between rodents and humans, their levels of brain anatomy and processing of neuronal signals share relatively high similarities. The linkage between NMS and visceral hypersensitivity in adult

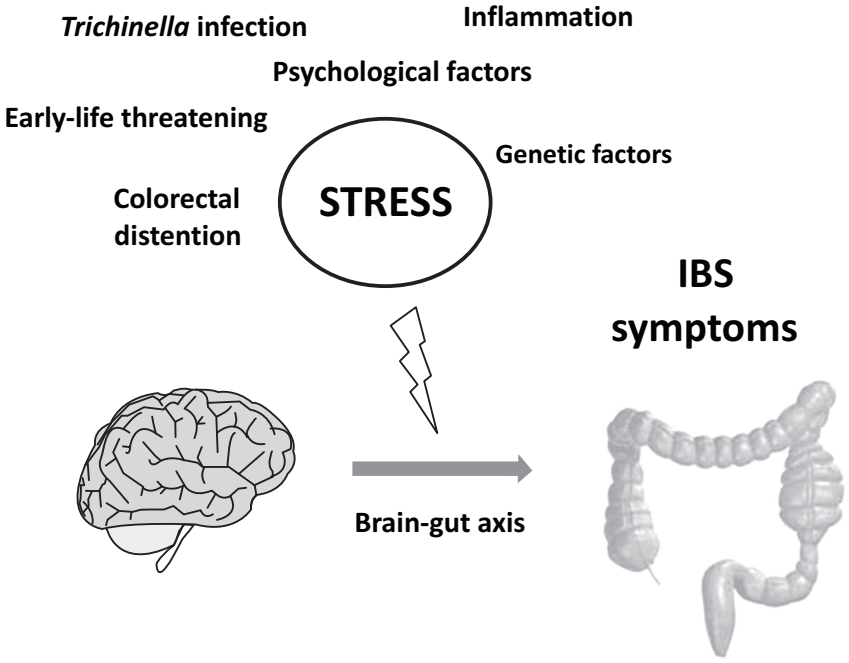

Fig. (1). Schematic presentation of the possible stressors for IBS patients.

life of rodents had been proposed by several research groups [51, 52]. Their findings generally indicated that the pain thresholds of the NMS rodents were remarkably lowered and their gut permeability was significantly altered when compared to the normal controls, meaning that they were more susceptible to pain and/or stressors. Importantly, expression level of nerve growth factor (NGF), which plays important roles in proliferation and differentiation of neurons, was elevated in spinal cords and colonic tissues of NMS rats that suffered from visceral hyperalgesia [53]. To this end, enteric nervous system, being the neural network of the intestines, markedly modulates the motor functions of the viscera. Moreover, corticotropin-releasing factor (CRF), a 41-amino acid neuropeptide, had been demonstrated to play a major neuroendocrine role in the activation of the HPA axis reactivity during visceral response to stressors [54, 55]. These findings have provided explanations to the imbalance of autonomic nervous system reactivity being observed in IBS patients [56, 57]. These results collectively strengthen the correlation between stress and bowel activities, implicating a close communication between the nervous systems and the gastrointestinal tract. The NMS model allows a better understanding of the pathophysiology of IBS caused by early life stress.

Colorectal Distention

Increased sensitivity to colorectal distention has been observed in IBS patients as a common manifestation of the disease. In fact, colorectal distention is considered as a kind of mechanical stimulation or noxious visceral challenge used to induce visceral hyperalgesia and the associated visceral pain [58]. This experimental procedure is widely accepted and highly reproducible for the assessment of visceral hypersensitivity in both clinical and preclinical trials. Similar to other sensation tests of hollow organs of the viscera, an inflatable balloon is used as the colorectal distending stimulus and is inserted into the colorectal cavity of the experimental human or animal subjects. To establish colorectal distention in non-human animals, *e.g.* rodents under anesthesia, an inflatable balloon is inserted into their descending colon and rectum. The inserted balloon is then inflated with compressed air while the pressure inside the balloon is monitored with a sphygmomanometer. Although the stimulus can be applied as either a constant volume or a constant pressure, the constant pressure distention had been demonstrated to be more reliable and reproducible in human trials [59]. The pain threshold of the experimental subject is determined after receiving colorectal distention at different time points [60]. Response magnitude and the slope of stimulus-response function are often assessed. Furthermore, colorectal distention is recognized to elicit visceromotor reflex actions, which can be measured in terms of electromyographic (EMG) recordings [61]. Henceforth, comparative examinations of electrophysiological as well as behavioral responses are often performed upon colorectal distention in research studies. With the aid of such setup, the effects of therapeutics can be evaluated according to the visceromotor responses or visceral nociception of the experimental subjects whereas components of the visceral pain pathway can be identified [62]. However, the main concern of this visceral pain model is the construction of the distending apparatus. For instance, the material, length and diameter of the balloon as well as the size of the corresponding catheter tubing may greatly affect the resistance to distention. The assembly and/or the placement of the distending apparatus may also contribute to some experimental errors. Nevertheless, the responses to colorectal distention are readily graded and quantifiable, and had been characterized and replicated by many laboratories. Collectively, an animal model of colorectal distention is regarded as an appropriate and useful tool in the study of pharmacological effects and the underlying visceral pain mechanisms of the central and peripheral nervous systems. This robust behavioral model also provides an approach explaining, at least partially, the brain-gut connection.

Restraint Stress

A restraint stress experiment is known to cause anxiety in animals; however, it is only considered as a mild form of psychological stress. Rats are often restrained for 30 minutes to several hours a day. During the restraint period, they are not allowed to access to water or food. The absence of food and drink does not alter their metabolism in a significant manner as short-term starvation is not perceived as stressful, especially during the light cycle. Restraint stress can be introduced to animals in an acute or a chronic manner [63]. An acute stress can be performed for as short as 30 minutes whereas a chronic stress can be executed for several hours a day for a period up to 28 days. This stress model has been shown to induce anxiety- and depression-like behaviors, including IBS symptoms. For evaluation of the outcomes in this model, several parameters can be taken into account. For instance, the number of fecal pellets during the immobilization period is often counted. Grooming behavior as well as levels of plasma glucose, triglyceride and cholesterol are also monitored [64]. Neuronal markers such as brain-derived neurotrophic factor (BDNF) are regulators commonly affecting post-restraint stress. Level of brain monoamines including serotonin, norepinephrine and dopamine will be assessed in order to explore further into the HPA functions under restraint stress [65]. Nevertheless, the importance of neurogastroenterology has been implicated in the pathophysiology of IBS.

Water Avoidance Exposure

According to a number of research studies, when animals are exposed to water avoidance stress (WAS), they would develop visceral hypersensitivity. Therefore, WAS induction is a good model of psychological stress [66]. In this stress test, animals are often placed on a glass platform in the center of a basin filled with water up to 1 cm below the glass platform for as little as 30 min to as long as several hours a day, representing acute or chronic experiments, respectively. Body weight gain, defecation and visceral sensitivity following the WAS test are often taken as measurement of stress-induced colonic motility. Both acute and repeated WAS exposures could induce immediate visceral hyperalgesia regardless of gender [67]. Moreover, the molecular effects of this behavioral test had been shown to be comparable to those induced by surgical obstruction of

partial bladder outlet [68]. Impaired mucosal barrier had been observed in rats receiving chronic WAS treatment [69]. In addition, a recent study has demonstrated that the number of mast cells, particularly those co-expressed with protease-activated receptor 2, was remarkably increased in rats induced chronically with WAS [70]. Taken together, psychological stress is markedly correlated with alteration of GI motility and intestinal barrier functions.

Acetic Acid

According to some clinical studies, when comparing to the healthy controls, IBS patients exhibit higher levels of acetic acid, which is a short-chain fatty acid of the human bowel. In fact, endogenous acetic acid is produced by bacterial fermentation of non-digestible carbohydrates and some hexose oligomers including lactose, sorbitol and mannitol, which is rapidly absorbed by the GI mucosal epithelium. IBS patients with high levels of acetic acid had manifested with more severe symptoms of IBS [71]. For understanding of the role of acetic acid in the patho-physiology of IBS, intracolonic instillation of 0.5-5% acetic acid can be used to induce IBS-like symptoms in animals. This results in acute inflammation in the mucosa and submucosa of the treated colon and can be characterized by edema, hemorrhage, infiltration of immune cells and occasionally ulceration. In this chemical-induced model, typically, visceral hypersensitivity was developed in the absence of mucosal damage [72].

Trinitrobenzene Sulfonate

Trinitrobenzene sulfonate (TNBS) is a commonly used pro-inflammatory procedure involving enema administration of a hapten compound for induction of visceral hyperalgesia, and has been considered as a prime animal model of PI-IBS that would result in low-grade mucosal inflammation [73]. In the TNBS model, severe colonic transmural inflammation often develops, leading to decreased pain threshold and elevated magnitude of responses [74]. Interestingly, the reoccurrence of visceral hyperalgesia was found 16 weeks after the induction of inflammation in more than 20% of the animals, without signs of any significant microscopic inflammation [75]. The results from the PI-IBS studies implicate the pathophysiology of IBS that involves immuno-modulation of the brain and the motor system of the gut as a post-inflammatory event. Furthermore, Qin *et al*. demonstrated that the disturbances in sensorimotor activities of the gut would largely contribute to the development of hyperalgesia under PI-IBS condition [76]. From their study, upon the binding to the cognate receptors on gut neurons, serotonin (5-HT) secreted by the enterochromaffin cells was considered as a stimulator of gut motility in TNBS-induced rats [77]. In fact, 5-HT is an important neurotransmitter and paracrine signaling molecule, which is renowned for stimulating the acetylcholine release from cholinergic neurons [78]. 5-HT mediated colonic contractility involves activation of branches of the vagus and pelvic nerves as well as other sympathetic pathways [79]. Similar findings had also been reported by Spiller *et al*. that altered numbers of enteroendocrine cells were found in IBS patients with recurrent abdominal pain and diarrhea [80]. Again, growing evidence has suggested that the colon not only communicates with other GI organs, but also associates closely with both central and peripheral nervous systems, which are largely influenced by the bidirectional activities of the brain-gut axis. Besides diarrheal IBS, visceral hyperalgesia is also a major pathophysiological mechanism in the IBS-C subtype. Decreased level of serum 5-HT, lowered expression level of serotonin transporter (SERT) and reduced counts of enterochromaffin cells were observed in IBS-C, whereas altered visceromotor reflexes such as decreased frequency and consistency of defecation and higher susceptibility to abdominal pain had been reported [81, 82].

Trichinella Infection

A number of research studies have demonstrated the correlation between changes of intestinal motility patterns and low-grade intestinal inflammation, which can be easily induced in rodents by *Trichinella spiralis* infection [83, 84]. Thus, this model was identified as a form of post-infectious or PI-IBS. Other than *T. spiralis*, *T., pesudospiralis*, *T. britovi* and *T. nelson* are other common species of the genus *Trichinella* that can be found in many carnivores and omnivores including human. As reported in some clinical studies, there have been cases when patients having history of acute GI infection developed with IBS symptoms, which amount up to 30% [85, 86]. A prognostic study even showed that the majority of the PI-IBS patients are symptomatic for at least 6 years post- infection [87]. Nevertheless, the use of an anti-nerve growth factor antibody had been shown to prevent the development of gut dysmotility in *T. spiralis*-infected rats [88]. Such result clearly demonstrates a role of the nerve system in the viscera, and the powerful influence by neurotrophins.

Transgenic Mouse Model

Other than exogenous stressors, animals with defective genetic background had also been shown to spontaneously develop GI disorders. Mice with a deficiency in CRF1 had demonstrated with decreased colonic sensitivity [89] as CRF is the principal neuro-mediator for stress-induced autonomic, endocrine, behavioral as well as visceral responses [90]. The marked reduction of anxiety in CRF1-knockout mice indicates that CRF1 has played an important role in the development of a functional HPA axis and the associated behavioral alterations [89, 91]. SERT knockout mice were demonstrated with visceral hypersensitivity since they possessed an increased colonic level of serotonin [92] as SERT, being expressed by enterocytes and responsible for the reuptake or clearance of serontonin and the termination of the serotonergic signaling. The establishment of such transgenic line undoubtedly represents a powerful approach for functional study of the genetic aspect of IBS.

In many occasions, more than one stressor may be applied to subjects in the IBS experimental models. For instance, NMS puppies are often subject to colorectal distention tests for evaluating the impact of early-life stress in gut motility. Moreover, the colorectal distention tests are also given to PI-IBS animals for evaluation of the long-term impact of inflammation on visceral pain threshold [93]. Collectively, it should be noted that the neuroanatomy of

Table 2. Examples of commonly used mainstream remedies for IBS patients.

Compound(s)	Target System	Major Action(s)
Hyoscyamine / Zamifenacin	Anticholinergic drug	Relaxation of smooth muscles; reduces colonic motility
Darifenacin	Muscarinic type-3 receptor antagonist	Treats active bladder *via* blocking the acetylcholine activities
Loperamide	μ-opioid receptor agonist	Suppresses diarrheal symptoms
Fedotozine	κ-opioid receptor agonist	Exerts anti-nociceptive effect
Gabapentin / Pregabalin	GABAergic agent	Enhances the release of neurotransmitters
Dextofisopam / Tofisopam	Modulators of benzodiazepine receptor	Suppresses diarrheal symptoms
α-helical 9-41 / GW876008	CRF receptor antagonist	Reduces visceral pain and anxiety
Cilansetron / Alosetron	5-HT_3 antagonist	Suppresses diarrheal symptoms
Tegaserod / ATI-7505 / TD-5108	5-HT_4 agonist	Improves constipation symptoms
Paroxetine / Desipramine	Neurotransmitter reuptake inhibitor	Functions as anti-depressants
Lubiprostone	Chloride channel activator	Increases intestinal secretion and accelerates the colonic transit of stool
Alosetron	Anti-depressant	Relax intestinal muscles and decelerates the passage of stool

laboratory animals, particularly rodents, are commensurate with that of human. Therefore, these preclinical IBS animal models can be important tools for evaluating the efficacy of therapeutic approaches prior to their translation into treatment strategies for human. Moreover, they are practical in unveiling the molecular mechanisms underlying effective therapeutic approaches. In the following section, various contemporary treatment strategies in clinical studies on IBS patients have been reviewed.

CONTEMPORARY THERAPIES FOR IBS PATIENTS

It is believed that changes of dietary style may help to reduce symptoms in some IBS patients. Nevertheless, drug therapy often remains the mainstream approach for treatment of IBS, possibly through improvement of GI motor functions, relief of visceral hypersensitivity and visceral pain from bowel spasm, attenuation of autonomic dysfunctions and suppression of mucosal immune activation [94]. Novel treatment modalities such as GABAergic agents and benzodiazepine receptor modulators have been employed in current practice and clinical trials [95]. A brief summary of the modern treatment approaches for IBS has been provided in Table **2**.

Anticholinergics

Anticholinergic drugs including hyoscyamine (*e.g.* Cystospaz®, Hyomax® and Levsin®) and dicyclomine (Bentyl®) have been served as pain-killers and relaxants of the GI smooth muscles for the relief of IBS symptoms. In fact, they are mainly used to reduce the motility of the stomach and intestines as well as secretion of gastric acid. Another class of anticholinergics is classified as anti-flatulent, for instance, simethicone, phazyme and mylicon, which are responsible for the reduction of intestinal or gastric gases. However, use of anticholinergic drugs had been well known for undesirable effects such as dry mouth and tachycardia. Muscarinic receptor antagonist, for example, zamifenacin had been demonstrated to effectively reduce colonic motility in patients with IBS with minimal side effects [96]. Lately, newer types of muscarinic receptor antagonists including darifenacin and solifenacin have been used to treat sensitive bladder owing to their specificity on blocking the acetylcholine-mediated action on muscarinic M3 receptors [97].

Opioids

Generally, opioids cause constipation. Morphine and codeine are the commonly used opiates that are reserved for more refractory cases of diarrheal conditions [98]. However, concerns about drug dependency with long-term opioid use in pain control as well as the induction of narcotic bowel syndrome have been continually expressed. For better pain control and management of adverse effects, opioid rotation has been suggested. New classes of opioid analgesics have been developed for targeting specific peripheral receptors only. The three major classes of opioid receptors μ, σ and κ are activated for various neural reactions with subtle differences. In general, the activation of these opioid receptors elicits analgesic effects [99, 100]. The anti-diarrheal agent loperamide (Imodium®) is an agonist acting on the μ-opioid receptors for suppressing diarrheal symptoms, and is recommended as first-line therapy [101]. Conversely, alvimopan is another μ-opioid receptor antagonist that accelerates colonic transit, whereas it can be used to treat constipation and/or postoperative ileus [102]. In some clinical trials, fedotozine and asimadoline are selective κ-opioid receptor agonists that exerted anti-nociceptive effects and increased threshold of perception for colonic distention in IBS patients, resulting in reduced visceral hypersensitivity in the subjects [101, 103, 104]. On the other hand, psyllium powder (Metamucil®) is used as a fiber laxative to treat IBS patients with constipation.

GABAergic Agents

Gabapentin serves as a conventional γ–aminobutyric acid (GABA)-modulating medication to reduce neuropathic pain and hyperalgesia by amplifying GABA transmission while inhibiting the α2σ subunit of the voltage-gated calcium channel in the central nervous system [105]. It can be used to control or prevent seizures in patients with epilepsy and is often prescribed as an anticonvulsant to treat IBS-D patients. It was first approved by the US Food and Drug Administration (FDA) in 1993 [106]. Pregabalin is another example of GABAergic agent being approved by the FDA in 2005 that has been used to treat rectal sensitivity by enhancing the release of neurotransmitters including glutamine and nonadrenaline and increasing the activity of glutamic acid decarboxylase. Pregabalin has been actively used as an IBS-D medication [107].

2,3-Benzodiazepine Receptor Modulators

Benzodiazepines are well known to enhance the effects of GABA through interaction with the benzodiazepine receptors, the allosteric modulatory sites adjacent to GABA-A receptors. Tofisopam is the most classical modulator of this kind and was approved to treat anxiety, menopause and IBS symptoms in many countries for a long time. Dextofisopam has been considered as the advanced form of tofisopam that can be used to treat IBS-D and IBS-A patients in some clinical trials to improve the consistency and frequency of colonic movements [108]. Although not much mechanistic studies had been undertaken in human, several research studies showed the effect of dextofisopam on the relief of pain reception being mediated *via* the benzodiazepine receptors of the central nervous system of rodents rather than directly acting on GABA receptors [109, 110]. Nevertheless, dextofisopam exerted significant improvement of IBS symptoms during the initial period of medication, of which its benefits had been reported to vanish in longer treatment times [108]. The development of tachyphylaxis is however a major concern for dextofisopam.

Corticoids

As described earlier, CRF is an important neuroendocrine hormone of the central nervous system, particularly for the activation of the HPA axis reactivity in visceral response to stressors. It had been reported that stress-associated up-regulation of CRF signaling enhanced gastrointestinal motility in rodents, and such up-regulation was repressed by the administration of CRF-1 receptor inhibitor [111]. Typically, CRF signaling is believed to be mediated *via* two subtypes of receptors, CRF-1 and CRF-2. Hence, the nonselective CRF receptor antagonist α-helical 9-41 had been administered to reduce visceral pain and anxiety in IBS patients [112], whereas specific CRF1 receptor antagonists, for instance, pexacerafont and GW876008 are still under development for potential clinical use [113].

Serotonergic Drugs

Serotonin, also known as 5-hydroxytryptamine (5-HT), is a principal neurotransmitter of the enteric nervous system as well as the brain-gut axis. Upon binding to its cognate receptor subtypes including 5-HT_{1-7}, multiple enteric functions such as motility, sensory perception and secretion are elicited [114]. 5-HT_3 antagonists and 5-HT_4 agonists had demonstrated with the most promising effects in the serotonergic category for treating the global symptoms of IBS. Selective 5-HT_3 antagonist cilansetron and alosetron had shown to be effective treatments in IBS-D patients [115, 116]. However, the risk of ischemic colitis is yet a concern in the administration of 5-HT_3 antagonists. In this regard, alosetron had once been approved by FDA, but was then temporarily withdrawn from the US market, while only being allowed to be marketable under a restricted label due to safety concern. Cilansetron, a more advanced form of alosetron, is well tolerated by both males and females, with only constipation as the major adverse event, waiting to be approved by FDA. On the contrary, the partial 5-HT_4 agonist tegaserod has been widely used to treat females with IBS-C symptoms in clinical trials. Nonetheless, events of ischemic colitis had still been reported after tegaserod treatment in some patients on vasoconstrictors, although the incidence was relatively lower than that caused by the 5-HT_3 antagonists. The efficacy of the new 5-HT_4 agonists such as ATI-7505, prucalopride and TD-5108 had been studied in large-scale randomized control clinical trials, with no adverse cardiovascular side effects being reported so far [117, 118]. Last but not least, according to a number of meta-analysis, serotonergic agents targeting the brain-gut axis are also collectively considered as effective treatments of IBS [119].

Antidepressants

Antidepressants are occasionally needed for treating IBS patients with IBS-associated psychiatric problems such as anxiety, negative mood, somatoform disorders and altered visceral sensation. These antidepressants are often neurotransmitter reuptake inhibitors such as paroxetine, desipramine and venlafaxine. There are two specific drugs for treating IBS as a last resort and must be used cautiously only when all other medications failed. Lubiprostone (Amitiza®), a selective chloride channel activator, is principally used as a laxative agent in patients with severe constipation as it increases secretion and fluid in the intestines and accelerates the colonic transit of stool. Side effects such as diarrhea and nausea may be associated, however they are often mild and transient. This drug was approved by the FDA in January 2006 [120]. Alternatively, Alosetron (Lotronex®) aims to relax the intestinal muscles and to decelerate the passage of stool. It was approved with restriction to be used only in females with severe diarrhea conditions, and is contraindicated in males.

Probiotics for IBS

In the recent decades, a number of clinical studies demonstrated that certain populations of gut microbiota are highly correlated to specific IBS co-morbidities, which are implied by the significant improvement of GI functions after antibiotic and/or probiotic treatments [121, 122]. When the commensal intestinal microflora of mice was disturbed by treatment of non-absorbable antibiotics such as neomycin and rifaximin, visceral hypersensitivity would be developed

[123]. On the other hand, unlike antibiotics, probiotics are live microorganisms in our dietary food such as yogurt, dairy products and medicines that may help to maintain healthy bowels. IBS patients had demonstrated to contain lower levels of certain beneficial intestinal bacterial species including *Bacteroides*, *Acinetobacter* and *Bifidobacterium* but increased levels of other enteropathogenic species such as *Clostridium, Proteobacteria* and *Firmicutes* in the stool. Many of the probiotics used in current therapies belong to the *Lactobacillus* spp. and *Bifidobacterium* spp. [124]. Generally, the number of microbes in the small intestine is not as high as that in the colon, however small bowel bacterial overgrowth (SIBO) develops when the normal growing condition is distorted. Symptoms of SIBO are similar to those of IBS such as diarrhea, constipation, bloating and abdominal pain. According to some animal studies, altered composition of gut microbiota in rats during neonatal period had been shown to have long-term impact on the development of their enteric nervous system as well as nociceptive pathways [125]. Therefore, gut microbiota has been suggested as a therapeutic target of visceral hypersensitivity and IBS. Microbiota manipulation may be novel strategies for treatment as well as prevention of IBS [126].

Prebiotics for IBS

In addition to the probiotic supplements, an increasing body of evidence suggests that prebiotics are also novel strategies for IBS patients. Prebiotics are commonly referred to a special form of dietary fibers that are found in many fruits and vegetables as the non-digestible food ingredients [127]. Literally speaking, as they are not living materials, they will not be affected by heat, cold, acid or duration and are resistant to a large number of digestive enzymes. They could promote the growth of beneficial bacteria in the gut while stifling those disease-causing pathogens [128, 129]. More data from randomized controlled clinical trials are needed for the thorough evaluation of the effective beneficial effects of prebiotic treatment on IBS patients due to the scarce number of clinical trials [126].

FUTURE STRATEGIES

The pathophysiology of IBS is multifactorial. Use of complementary and alternative medicine has increasing popularity among IBS patients. A recent study has suggested that a mixture of β-glucan, inositol and digestive enzymes notably improved some IBS-related symptoms including abdominal pain, bloating and flatulence in IBS patients [130]. In fact, a number of alternative treatment approaches have been used for alleviation of abdominal discomfort, for instance, acupuncture, oils and supplements, herbal formulations and functional peptides, while sex hormones, interpersonal psychotherapy, cognitive behavioral therapy and progressive muscle relaxation exercises may also be considered.

Acupuncture for IBS

Acupuncture is an important treatment approach in the practice of Traditional Chinese Medicine (TCM), and becomes a popular alternative strategy in the therapy of IBS and associated conditions such as chronic abdominal pain and muscular injury. Acupuncture procedures involve insertion of extremely thin needles through the skin at certain strategic locations ("acupoints") along functional "meridian" pathways to alleviate the symptoms of IBS, the acupuncture technique can be used to restore the harmony between the "*spleen*" and "*liver*", two hypothetical disease-related systems according to TCM principle. In addition, acupuncture is also known to be rooted to the perception of pain in the central nervous system. According to our ongoing clinical practice, the use of acupuncture has been an effective approach to resolve abdominal pain in IBS patients. The effect of acupuncture on IBS patients had been examined in a large-scale randomized control trial. Unfortunately, no significant improvement of IBS symptoms was observed in patients given with the acupuncture treatment [131]. Therefore, we are in the process of establishing a more systematic clinical trial on acupuncture in IBS patients at the university clinics, aiming to achieve a more promising result.

Oils and Supplements for IBS

Among several complementary and alternative approaches, natural remedies have an obvious appeal. Historically, peppermint oil had been using as a natural remedy for the relief of IBS symptoms for a long time. Several large-scale studies have shown that the enteric coated peppermint capsules notably reduced IBS symptoms, particularly for the relief of abdominal pain, although some of the effects were considered transient [132, 133]. Besides peppermint, olive oil and coconut oil are good natural alternatives. On the other hand, supplements such as curcumin have helped IBS sufferers to have improved bowel movements [134]. Indeed, curcumin contained in standard Indian spicy herbs had demonstrated to possess diuretic, stimulant and antispasmodic properties. Furthermore, other smooth-muscle relaxing herbs such as lemon balm, lavender balm and chamomile have also been used to stabilize the nerves, so as to ameliorate the stress-related GI dysfunctions. Moreover, wild yam, licorice and linseed have been suggested to be used as laxatives for constipation-predominant IBS sufferers, whereas rose, tormentil and agrimony are suggested for treating diarrheal conditions.

Herbal Formulations and Compounds for IBS

The use of single herbs or herbal formulation is common among IBS patients worldwide. Owing to the lack of scientific evidence available that supported the use of many traditional herbal formula, their efficacies had been questionable despite the fact that they have been used as folk medicines for centuries. With the advancement in biotechnology, the standardization and quality of herbs as well as the evaluation techniques and assessment criteria not only allow elucidation of the active components of the herbal formulas, but also delineate their underlying molecular mechanisms. The Chinese herbal formula JCM-16021 has been shown to act *via* suppression of 5-HT activity, which could effectively reduce visceral hypersensitivity while restoring the visceral pain thresholds in rats with PI-IBS [77]. Apart from China, the use of Chinese herbal formulation had also been tested in other Western countries as early as in the 1990s. In an Australian clinical trial, IBS patients

receiving a 20-herb formula had resulted in a significantly improved disease activity score when compared with the placebo group, with no major adverse effect being reported [135]. However, the molecular mechanisms for such effective formulations are yet to be unveiled.

Functional Peptides

Peptides play a vital role as mediators of key biological functions due to their unique properties concerning efficacy, selectivity, specificity and low toxicity. Functional peptides have the potential to be developed as therapeutic agents in treating cardiovascular disease, infectious diseases, immunological disorders, gastrointestinal dysfunction and cancers [136]. A stable gastric pentadecapeptide BPC157 has been established as an anti-ulcer agent and was proven to be effective in IBD clinical trials by facilitating wound healing [137]. It was then discovered that BPC157 could exert GI protection and restore normal physiological functions in different sections of the intestine. Other than the known anti-lesion and structural repairing functions in multiple organs, its diversified activities range from restoration of normal sphincter pressure, maintenance of visceral vasculature integrity to mesenteric neuroprotection. Evidence has shown that the beneficial effects of BPC157 in the gut could improve patients' QoL, resulting in constant weight gain in patients of short-bowel syndrome following operation. It has been suggested that BPC157 could improve the effectiveness of therapy on GI disorders. The symptoms associated with IBS and related brain-gut neuronal dysfunction may also be modulated by this novel agent.

Sex Hormone Therapy

Both epidemiological studies and animal experimentation have implicated stress as a trigger to IBS symptoms (first onset or exacerbation), which involves activation of the CRF signaling pathways [138]. In the Western society, it is known that IBS is predominant in female, of which similar discrepancy has not been clearly shown in Eastern countries. Besides, female IBS patients are more likely to report constipation, bloating, severe abdominal pain, feeling of incomplete evacuation, as well as the more distinctive complaints of joint and muscle pain when compared with male patients. Besides, sex differences in stress response of the HPA axis, stress-induced neuroimmune interactions and estrogen interactions with CRF signaling system have been increasingly recognized [139]. Hence, by understanding the role of sex hormones in the pathogenesis of IBS, therapeutic approach aiming to suppress ovarian steroidogenesis has been considered. However, although gender differences in response to treatment modalities seem prevalent, the therapeutic approach to IBS patients in both sexes is quite similar so far. More evidences have indicated a protective role of androgens in pain modulation and anti-inflammatory properties of testosterone that may inhibit the development of visceral hyperalgesia. In fact, it is interesting to know that there are sex differences in the gut microbiome, which would drive hormone-dependent regulation of autoimmunity. Detail investigation on the modulation of the brain-gut-microbiota axis is needed in order to establish a more effective sex-tailored therapeutic approach in IBS.

Interpersonal Psychotherapy

A growing body of evidence suggests that psychodynamic therapeutic approaches are helpful in the reduction of IBS symptoms [140, 141]. Psychotherapy is commonly referred to an insight-oriented approach targeting on the management of interpersonal relationship. According to the outcomes of several clinical trials, other than GI discomfort conditions, the physical and emotional status of IBS patients receiving interpersonal psychotherapy had also manifested with significant improvement [141]. As a result, fewer outpatient visits to gastroenterologists had been observed. The use of this kind of therapy is believed to reduce the body's sensitivity to the external or internal stressors *via* the modulation of mental status. This category of treatment, once again, undoubtedly explains the importance of brain-gut axis activity in the pathogenesis of IBS.

Cognitive Behavioral Therapy

Gut-directed hypnotherapy is a new approach of cognitive behavioral therapy, being identified as a viable treatment of IBS [142, 143]. The execution of hypnotherapy often involves individual or group sessions over weeks or months. However, the effectiveness of such treatment largely depends on the actual performance and experience of the hypnotherapy practitioners, who are familiar with both hypnosis as well as the complicated functional GI disorders. A number of clinical studies have shown that primary IBS symptoms such as diarrhea, constipation, abdominal pain, bloating as well as the overall QoL could be improved in patients receiving gut-focused hypnotherapy [144, 145]. When combined with other conventional treatment approaches, the improvement on bowel habit abnormality became even more promising. Cognitive behavioral therapy may provide some degree of symptomatic and physiological benefits.

Progressive Muscle Relaxation Exercises

Progressive muscle relaxation exercises are used to reduce the sensitivity of the body's viscera [146, 147]. Relaxation exercises such as breathing, yoga, meditation, Tai Chi, bathing are commonly used [148, 149]. These types of exercises could be helpful to relief general stress condition and the associated IBS symptoms. More importantly, these exercises are particularly attractive as they have no major adverse effects.

CONCLUDING REMARKS

When facing with difficulty in the management of chronic disorders such as IBS, complementary and alternative approaches are definitely helpful to sufferers. To cope with the alteration of activity in the brain-gut axis, therapies that provide symptomatic, psychological and physiological benefits should be sought. The mainstream drug therapy and conventional remedies are sometimes not satisfactory in the relief of the multifactorial symptoms of IBS. Hence, IBS patients start to seek for effective alternative therapies, especially those without major side effects. Nevertheless, the efficacies and safety of these

complementary and alternative approaches may yet to be proven by more scientific evidences.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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